Deltavision OMX (Structured Illumination Microscopy)

I. Starting the Deltavision OMX

1. Sign in to the log book
2. Turn on the lasers in the laser enclosure box (shorter black box).
   A. Turn key to right.
   B. Flip switches for lasers needed. There are five — 405, 488, 514, 568, 642. Light outside of laser cabinet will change from green to red when lasers are on.
3. Log into the PC computer (left) using your VUNetID and password. You should not have to log into the Linux computer (right). If you do, the username is “worx” and the password is “OMX050SI”.

II. Starting the OMX and Softworx software

1. Start OMX software on PC computer.
2. Start Softworx software on Linux computer.
3. When you open the OMX software, the screen should look like this.
III. Choosing OMX settings

1. In OMX software, set Image Mode to Sequential.

2. Choose Light Path as SI for structured illumination.

3. Choose Size of 512x512 resolution.


5. Select channels for your application. Click box (A, B, C, D) to turn on (blue letters). Choose fluorophore name from the drop down list next to each blue letter.

6. Under each channel to be used, set the following 4 things.
   
   A. Set Mode to Med 95 MHz.
   
   B. Set Exposure to 100 msec.
   
   C. Set Excitation wavelengths as appropriate for your fluorophores.
      
      DAPI=405
      FITC=488
      TRITC=568
      Cy5=642
   
   D. Set laser power (%T) to 10%.

7. Choose “escape 10000” under Z touchdown to raise stage up.
IV. Choosing your initial immersion oil

1. Determine which oil to use for your sample by choosing **Utilities** in the Softworx software (right computer) and choosing “Lens Information”.

2. Enter **Lens ID: 10612**


   A. Choose **Distance from Coverslip to Specimen (um)**. If cells are grown on coverslip and mounted on slide, choose 1.

   B. Set **Temperature** to 24. You can find the current temperature of the microscope enclosure and the room under Instrument and Temperature in the OMX software (left computer). Use temperature of microscope enclosure.

   C. Set **Coverslip Thickness (um)** to 170 for standard 1.5 coverslips.

   D. Choose your mounting media from pull-down menu. Prolong Gold is a good recommendation.

4. See **Recommended Refractive Index**. Often, oil 1.516 is a good start, and the usual range is 1.514-1.520. It is difficult to match the oil perfectly for each wavelength when imaging multiple colors. Choose the most important wavelength to optimize the oil or try to match 2 if you are doing 3 color imaging.

See SAMPLE PREPARATION notes on last page of guide.
V. Mounting the slide and finding focal plane

1. Ensure that your slide is clean and dry. It is very important that you clean off any oil from other microscopes.

2. Open door to microscope enclosure (taller black box) and add oil to objective (chosen in section IV).

3. Mount your slide upside down in the spring loaded slide holder (blue).

4. On left computer in OMX software, center slide. Use $d_X$ and $d_Y$ arrows to center slide over objective. Distance moved is in um to the right of $d_X$ and $d_Y$. The stage moves, and the objective does not move. If you want to move the stage toward you (objective relatively away from you on coverslip), then choose $d_Y$ arrow up. If you want to move the stage left (objective right on coverslip), then choose $d_X$ arrow left. To move quickly, estimate the distance needed and type in the number of um to move with each press of the arrow.

5. Position Z in range using Z touchdown. Choose touchdown from pull down menu and the stage will move to that position. Range is usually 3000-3500. If you do not have a Z recommendation, HeLaCS 3339 is a good start.

6. Choose one channel to image for locating sample. Turn off other channels. Click on camera button to take image. Look for out of focus objects and adjust accordingly by moving $d_Z$ by 10-20 um up and down until you get objects in focus.

7. Ultimate Focus (UF) or Spiral Mosaic (next page) can be used to find focal plane.
V. Mounting the slide and finding focal plane (continued)

8. Spiral Mosaic can be used to find focal plane.
   
   A. Choose one channel for spiral mosaic and uncheck others under **Light Parameters**. Set exposure time at 100 msec or less.
   
   B. Set square size at **XY max size FOVs** as 9x9 or 10x10. Set as single number (9).
   
   C. Click Start.
   
   D. When you find an out of focus spot, click **Go To Point** and click on desired square in mosaic.
   
   E. Make small adjustments with **dZ** arrows (1-5 um) to focus sample.
   
   F. If you still see nothing, go up and down 10 or 20 in dZ or try another spiral mosaic in a new region.

9. Three examples are shown for out of focus light.

   A. Very little of the desired sample is visible here, but you can see an out of focus object. Look for lighter and darker regions in the different areas. If you do not see dramatic changes in the max value as you change dZ, there is no important image in that area.

   B. By moving in Z (up or down), an out of focus cell is now visible.

   C. By continuing the same direction in Z, the cell is now in focus.
VI. Optimizing your immersion oil

1. Find a focal point in your sample. You should have just completed this in Section V. For optimizing your immersion oil, small, punctate spots of fluorescence are best.

2. In the OMX software, click the dZ arrow up in 1 um increments for least 3 slices. Do the same for dZ with the down arrow.

3. If the images are identical up and down, then your current oil is correct. If you see airy rings (concentric circles around your spot of interest), then you need to adjust your oil. If you see airy rings when you go **UP in Z**, then you need to go **DOWN in oil**. If you see airy rings when you go **DOWN in Z**, then you need to go **UP in oil**. **NOTE:** If you are looking at your collected image in Softworx, this relationship is reversed.

4. If you need to change the oil, remove slide, clean slide well with Sparkle, clean objective well with lens paper, and start over with new oil.

**NOTE:** It may be difficult to find an oil that is perfect for every wavelength. Choose the most important channel or an intermediate wavelength.
VII. Setting parameters for image acquisition

1. Once you find your region of interest, you can center your sample with bullseye button. Click on bullseye icon, then click on image point that you wish to be center of image.

2. Under **Light Parameters**, adjust exposure time and laser power (%T) for each sample to optimize range. Minimum will not change much, but you want the maximum to be similar between all channels. Set each channel independently by turning off other channels. A good start is 3,000-7,000 maximum for each channel. If you have a good signal with low laser power and low exposure, you can adjust range up to 10,000 or even 20,000 maximum. To avoid saturation, stay below the maximum of 32,767.

3. Set Z limits for Z stack. Go up and down with dZ in steps of 0.25 or 0.5 um. The smallest Z stack possible is going to give the best final, processed image. The minimum number of slices for reconstruction is 6. Because the optical slice is set at 0.125 um, the minimum Z stack thickness is 0.75 um. A good range is 1-2 um total for Z stack.

4. Go up in Z by using the dZ up arrow. **Mark top.** Go down in Z by using the dZ down arrow. **Mark bottom.** After both are set, **visit top.** You will confirm this point (top, middle, bottom) in the next section.

**NOTE:** As with any fluorescence imaging, there are tradeoffs between maximum signal intensity, laser power, and exposure time with regard to signal and to photobleaching. The initial setup suggestions of 100 msec, 10% laser power, and maximum intensity of 10,000 are good starting points. Optimizing these values for your sample will be discussed during your training.
VIII. Acquiring image

1. Click on **Experiment** tab.  
2. Choose **SI** under Experiment Type.  
3. Check **Focus point when scan starts**. Make sure this matches your visit point from the previous section. If not, visit this point.  
4. Click on **Get thickness** to set Z stack. Do not change 0.125 um optical spacing.  
5. Check that all channels to be imaged are turned on (blue).  
6. Click **Save As** to choose location to save file and to name file. Save in Data1 in folder with your name or your lab name. **DO NOT USE ANY SPACES OR DASHES IN YOUR FILE NAME OR FOLDER NAME.** Underscore is fine for separating words.  
7. **RUN.**
IX. SIM processing

1. Open data file (.dv) file in Softworx program. File is in Data1 folder on desktop of right computer.

2. First, do SI Reconstruction. Under Process, choose **OMX SI Reconstruction**. Add file to Input. You can do this by dragging the file name from the open Data1 folder or by dragging the window number from the image window (here it is number 1). Set two things.

   A. For **Use Channel-Specific OTFs**, click on **Set Up OTFs**, and choose BGR. After clicking on the “unassigned” button, click OK.

   B. For **Use Channel-Specific k0 Angles**, click on **Set Up K0 Angles**, and choose BGR. This should already be done. Click OK. Click “Do It” and, image will automatically be processed.

3. Second, find _SIR.dv file in your folder in Data1 and do image registration. Under Process, choose **OMX Image Registration**. Drag _SIR.dv file name into Input. Set **Image Source Drawer** as BGR. Click “Do It”. Final image is _SIR_ALX.dv.

**NOTE**: Reconstruction and Registration must be done in the same image session because of camera registration.
IX. SIM processing (continued)

NOTE: Reconstruction and Registration can be done together in batch processing. Also, multiple images can be processed in batch processing.

A. Choose Task Builder under Process. Drag files to process into Image Files to Process box. You can do this by dragging the window number in the open image or by dragging the file name from Explorer.

B. Under Processing Tasks, choose OMX SI Reconstruction.

C. Next, click “Add” and then choose OMX Image Registration.

D. Set both to BGR as detailed on previous page.

E. Confirm that Automatically Run the Queue is checked.

F. Click Submit to Queue.
X. Post-acquisition processing

1. If desired, max projection can be made. Under View, choose Quick Projection. Drag and drop file name into input.

2. If desired, 3D reconstruction can be created. Under View, choose Volume Viewer. Click on Interactive at the bottom. Be sure to choose channels and quality.

3. NOTE: Pseudocolors are not standard if imaging three color RGB. Colors default to alphabetical (BGR). This must be adjusted for every image and for every new window. In the image window under View, choose Select Image Colors and adjust to desired color combination.

4. Images can be saved as TIF. Choose File (in image window), Save As Tif. Under Destination Computer, choose PC or Mac. Choose Output Size as 24 RGB.
Sample Preparation

1. For best results, grow cells directly on coverslip and then mount the coverslip on the slide.

2. Coverslips should be 1.5 (0.17 um). If thickness is not precisely 0.17 um, image quality may suffer.

3. Only one coverslip per slide should be mounted and it should be centered on the slide.

4. Coverslips should be clean and dry with NO oil from other scopes.

5. Avoid DAPI in mounting media. DAPI gives too much background fluorescence.

6. Prolong Gold from Life Technologies (no DAPI) is a good mounting media.

7. The maximum distance from the coverslip to the sample should not exceed 20 um for best results.

XI. Ending session

1. Transfer data to your personal folder on CISRSTORE or other storage. For instructions for accessing and mapping your folder in the CISRSTORE drive, see the FAQ page on the CISR website. **NOTE: Files are very large!**

2. Remove slide and clean oil from objective with lens paper.

3. Choose Z touchdown of “escape 10000” to bring stage up away from lens.

4. Check calendar for next user.

5. If not one is coming on for 30 minutes, log out of computer and turn lasers off with switches and then single key. **DO NOT TURN OFF “MAIN POWER” SWITCH.**

Contact information

- After completion of training by a CISR staff member, you will be granted access to the online scheduling calendar and card access to the room.
- CISR website
  http://cisrweb.mc.vanderbilt.edu
- Contact: CISR Staff in
  742 Light Hall — 3-3750
  Jenny Schafer
  Carol Ann Bonner
  Bob Matthews
  Sam Wells
  4155 MRB III — 6-3706
  Sean Schaffer

You too will be creating beautiful images in no time!

Single cilium projecting from normal human kidney costained for acetylated tubulin (green) and Arl13b (red). Image courtesy of Lynne Lapierre, PhD

HeLa cell expressing GFP-Rab11-FIP1A costained for Rab4 (red) and Rab11a (blue) to mark vesicles and tubules of the recycling system. Scale bar is 5 um. Image courtesy of Jenny Schafer, PhD

H/K-ATPase (green) and ezrin (red) in normal parietal cells. Left: Parietal cells with normal resting pool of H/K-ATPase. Right: Parietal cells with normal H/K-ATPase distributed to canalicular surface. Scale bar is 5 um. Image courtesy of Byron Knowles, PhD

Acknowledgment:

Our funding depends upon your citing the Cell Imaging Shared Resource (CISR) when you publish data obtained with equipment or services from CISR. This includes images from microscopes, training in the use of software, consulting on data analysis, etc. The minimum acceptable acknowledgment should read:

“Experiments/data analysis/presentation [include what you use]” were performed in part through the use of the VUMC Cell Imaging Shared Resource, (supported by NIH grants CA68485, DK20593, DK58404, DK59637 and EY08126).

Please let us know when you publish and, if possible, send up a reprint of the paper. This is how we justify our existence.